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The Effect of Apricot Tree Gum on the Quality of Frozen and Melted Ram Sperm in the Breeding Season

Research Article

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ABSTRACT

The adding gum of some fruit trees preserves the quality of semen during the freezing and thawing process. The aim of this study was to investigate the antioxidant effects as well as the sugars in apricot tree gum to increase the quality of ram semen during the freezing, storage and thawing process**.** In this study, 4 treatments were used. The control group was without any apricot and the three treated groups were pure and sterilized apricot gum with concentrations of 10, 15 and 20 mg/mL in a tris-based diluent. Semen collection was performed twice a week during the breeding season using artificial vagina, and sampling was replicated 5 times for each ram. After transferring the samples to the laboratory and diluting them to ensure that they meet the required standards. After cooling the straws in the refrigerator, they were placed in liquid nitrogen. Total motility, progressive motion, survival, abnormality and membrane integrity assessment were measured in the first, 15th, 30th, 45th and 60th days after freezing. Plasma malondialdehyde levels were also assessed after thawing on day 60. The three treated groups of 10, 15 and 20 mg/mL in diluent were significantly different from the control group in total motility, progressive motion, survival, abnormality, membrane integrity, and malondialdehyde levels ($P<0.05$). There was no significant difference among the treatments of 10, 15 and 20 mg/mL in diluent in all measured traits ($P>0.05$). However, the treatment of 20 mg/mL had the highest total motility, membrane viability and integrity, and the lowest malignancy and malondialdehyde, and groups of 10 and 15 mg/mL had the highest progressive motion ($P<0.05$). Considering the results, use of apricot tree gum can improve the quality of sperm cryopreservation.

KEY WORDS apricot gum, freezing, malondialdehyde, sperm, thiobarbituric acid.

INTRODUCTION

The sheep industry has not been able to utilize many of the assisted reproductive technologies (ART) in general and AI in particular, as other livestock industries, due to inefficiencies in collecting, freezing and inseminating frozen ram semen (Soltanpour and Moghaddam, 2013). Ram semen is more sensitive to cold shock during freezing than other species such as bulls and rabbits (Taghilou *et al*. 2017).

In particular, ram sperm are very sensitive to freezing due to their low phospholipid to cholesterol ratio (Juyena and Stelletta, 2012). The plasma membrane of sperm is the main site of injury during the freezing and thawing process (Anand *et al*. 2017). The plasma membrane of sperm is very sensitive to lipid peroxidation due to its high content of polyunsaturated fatty acids (Sterbenc *et al*. 2014). Freezing of the semen in liquid nitrogen enable long term storage of fertile spermatozoa from different animal species (Sterbenc *et al*. 2014) and controls the prevalence of many infectious diseases (Juyena and Stelletta, 2012), but it causes partial irreversible damage to the sperm cells. This has been attributed to sperm cold shock, oxidative stress,

sperm membrane modification, cryoprotectant toxicity, intracellular ice crystal formation, and fluctuations in osmotic pressure (Soltanpour and Moghaddam, 2013), and also cause reduction in motility and morphological integrity of spermatozoa. These changes may be contributed to the accumulation of the toxic products of metabolism, mainly of reactive oxygen species (ROS) formed through lipid peroxidation of the membranes of spermatozoa (Dolti *et al*. 2016). Three regions of frozen semen in the python that are affected. The "ice front" zone results from rapid freezing, which occurs on the outer edge of the straw. The majority of sperm in this zone sustain damage. The "optimum" zone is a region of maximum sperm survival because the ice crystal-solute conditions are compatible with viability. The "solute" zone results from increasing concentration of salts. Spermatozoa in this area are damaged owing to dehydration (Morrow, 1986). Mammalian sperm is a redox active cell that spontaneously produces ROS. This production begins as soon as the sperm leaves the epididymis (Aitken, 2000). ROS attack and sperm dysfunction occur with a direct impact on the sperm oxidative defense system and result in decreased motility, mitochondrial activity, decreased plasma membrane integrity and induction of sperm apoptosis (Mehdipour *et al*. 2016). The most common forms of ROS, which affect sperm survival and function, include hydroxyl radicals (OH), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), peroxide radicals (ROO), and hypochlorite radicals (Makker *et al*. 2009). Hydrogen peroxide (OH) can cross the sperm membrane and enter its cytoplasm, inhibiting the activity of some enzymes, including glucose-6-phosphate dehydrogenase. This enzyme controls the entry of glucose into the hexose monophosphate pathway. This pathway normally produces reduced nicotinamide adenine dinucleotide phosphate (NADPH) for reductive reactions in the cell. Inhibition of the monophosphate hexose pathway reduces the production of NADPH, as a reducing regulator in the cell. Glutathione peroxidase is a major antioxidant enzyme in sperm that uses reduced glutathione to reduce ROS. By reducing the level of NADPH, due to the inhibition of glucose-6-phosphate dehydrogenase, it reduces the activity of glutathione peroxidase, as an antioxidant defense. Thus, the rate of peroxidation of sperm membrane phospholipids increases, resulting in a decrease in membrane fluidity. The result of these changes will be a reduction in sperm motility. Several products are produced during the lipid peroxidation process, the most important of which is malondialdehyde (MDA) (Khosrowbeygi *et al*. 2004). Malondialdehyde (MDA) is an end-product of enzyme and free radical-catalyzed lipid peroxidation of polyunsaturated fatty acids including arachidonic acid.

Circulating MDA is one of the commonly and widely used biomarkers of oxidative stress (Tsikas *et al*. 2016).

MDA can react with biological macromolecules (such as proteins and nucleic acids), change the fluidity and permeability of cell membranes and finally change the structure and function of the cell (Zhang *et al*. 2020). Diluents for freezing ram semen should have similar properties to diluents for fresh use of semen, they should be buffered against changes in pH and tonicity and contain an energy source (Soltanpour and Moghaddam, 2013). Natural mechanisms which protect spermatozoa against lipid peroxidation include various antioxidants and enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase (Sterbenc *et al*. 2014). Adding the supplementation of anti-oxidants to enhance sperm characteristics during the preservation of sperm cells is well known (El-Harairy *et al*. 2018).

Addition of antioxidants to sperm diluents increases antioxidant protection and improves sperm motility, mitochondrial activity and survival (Juyena and Stelletta, 2012). Herbal exudate gums normally secrete from bark, branch and fruit of trees due to their protection impact against mechanical damage or microbial attacks (Khorsha *et al*. 2016). Gums are a complex of polymeric structures contained mainly, as a monomer, pentose's, hexoses and uronic acids (Saniewski *et al*. 2002).

Apricot tree gum can be a good alternative to synthetic polysaccharides in various industrial aspects (Chichoyan, 2011). The polysaccharide gums represent one of the most abundant industrial raw materials and have been intensively applied because of their biocompatibility, renewability, nontoxicity, biosafety, biodegradability, sustainability, and capability of easy chemical alterations (Iravani, 2020). According to Rosik *et al*. (1971) apricot gums formed by *Cytospora cincta sacc* contained galactose, arabinose, xylose, mannose, glucuronic acid and 4-o-methyl-glucuronic acid. Polysaccharides of apricot gums contained two main carbohydrates: galactose and arabinose, with xylose as minor component (Saniewski *et al*. 2002). The chemical composition and physicochemical properties of apricot gum cultivated in various regions of Central Asia were first studied by Umansky *et al*. (1946). Gum, according to its data, includes glucuronic acid - up to 16%, galactose - up to 44%, arabinose - up to 41%; a mixture of protein substances does not exceed 0.6% (Chichoyan, 2011). According to the research of Samaei *et al*. (2017) apricot tree gum has a pH of 1% solution (weight-volume) 7.87, moisture 9.5 ± 0.13%, protein 1.38 ± 0.29%, ash 3.43 ± 0.01%, carbohydrates 85.69 ± 0 0.32% and no fat were obtained. Chemical analysis of apricot gum has already been performed by Lluveras-Tenorio *et al*. (2012).

They found total sugars (60%), galactose (43%), mannose (4%), arabinose (44%), xylose (7%) and rhamnose (1%) in apricot gum.

Also the results obtained in the research of Fathi *et al.* (2016) apricot gum contains carbohydrates 66/89%, uronic acids 10.47%, moisture 6.9%, protein 2.91%, ash 4%, fat 1.59%, monosaccharides including L-arabinose, D-galactose, xylose, mannose and rhamnose in molar ratios are respectively 41.52%, 23.72%, 17.82%, 14.40% and 2.54%. The results of the research of Zitko *et al.* (1965), apricot gum has a dry matter content of 89.3% and the molar ratios of xylose, L-arabinose and D-galactose are 8: 8: 1, respectively. Also, the molar ratio of galactose, arabinose and xylose in apricot gum obtained by Saniewski *et al.* (2002) was 14: 10: 1, respectively. Simple phenols-derived compounds are also present in apricot gum. The maximum recorded values for phenols include catechol's, hydroquinone's and pyrogallols amounds of which are respectively 7.58%, 4.27% and 5.69% (Babken *et al.* 2018). Phenolic compounds are a class of antioxidant agents acting as free radical terminators (Mahmoudi *et al.* 2010). Sugars play different roles in diluents. They provide energy during incubation, maintain a dilute osmotic pressure, and act as a cryoprotectant. The beneficial effect of sugar supplementation of the extender on the post-thaw viability of mammalian sperm cells have been reported in many studie (Yildiz *et al.* 2000). Preservatives used for cell freezing are classified into intracellular and extracellular preservatives. Internal protectors such as glycerol have low molecular weight and amphipathic properties and penetrate into the cell and are effective in reducing cell damage during slow freezing. Extracellular protectors work more by forming a coating around the cell and reducing the effect of dehydration during the freezing process (Motta *et al.* 2014). Impermeable protectors are mainly monosaccharides, oligosaccharides and polysaccharides. Some sugars cannot cross the plasma membrane and cause osmotic pressure, which causes loss of intracellular water and reduces the formation of intracellular ice. These sugars react with phospholipids in the plasma membrane and increase the survival of frozen sperm (Dolti *et al.* 2016). Sariözkan *et al.* (2012) reported that the use of sugars has a beneficial effect on maintaining motility, healthy morphology and preserving the structure of rat sperm DNA against cooling damage. Phenol and its derivatives are microbicidal or microbistatic depending on the concentration and temperature. Phenolic compounds produce proteins, and their main mode of action probably involves disruption of the bacterial membrane (Singleton and Sainsbury, 1981), which indirectly prevents the formation of ROS. Apricot gum due to its sugars, especially monosaccharides such as arabinose, galactose, xylose and mannose can maintain sperm energy during storage and protect the formation of intracellular ice crystals by dehydrating sperm cells. Due to its phenolic compounds, apricot gum can have antioxidant properties, prevent the formation of ROS directly and also indirectly prevent the formation of ROS with its antimicrobial properties.

MATERIALS AND METHODS

Preparation of apricot tree gum was collected from apricot trees located in Khalatpooshan research station of Tabriz University, East Azerbaijan province and stored in the refrigerator temperature. One gram of gum was solved in 10 ml of sterile distilled water and was sterilized using autoclave.

Apricot tree gum analysis

The results obtained from the analysis of apricot gum in the Advanced Physiology and Animal Nutrition Laboratory of Tabriz University are as follows in Table 1.

Table 1 Chemical compounds of apricot gum

Chemical compounds	Amount
Carbohydrate (%)	77.06
Uronic acids (%)	10.13
Protein (%)	2.95
Moisture (%)	5.9
Ash (%)	3.96
pH	7.39

Preparation of apricot gum

Apricot gum was ground by an electric grinder (model LM30214A, made in Iran) and the milled mixture was passed through a 50 mesh sieve to separate impurities and coarser grains. The resulting powder for purification steps inside the bag plastic zippers were placed. 3% suspension (weight-volume) of apricot gum was prepared in distilled water and stirred overnight at 25 ˚C with a magnetic stirrer. Then, to separate insoluble compounds and impurities, the suspension obtained from the fabric filter O was passed properly. Because the 3% (weight-volume) suspension of apricot kernel gum was not very thick, it was easily passed through a cloth filter. The filtered portion of the filter was dried by freezing dryer. To separate the polysaccharide portion of the gum, solution 2 percentage (weight-volume) was prepared from dried gum and three times its volume was added 96% ethanol. The resulting sediments were collected and after dissolving in water, they were dried again by freeze drying. And after passing through a sieve with 50 mesh to be stored in plastic bags for further testing. Purification efficiency was calculated according to the following formula by measuring the weight of the purified sample relative to the weight of the raw material (Rahimi *et al.* 2013).

Purification efficiency= (purified sample weight of apricot gum (g)/weight of apricot gum prototype) × 100

Determination of chemical composition of apricot gum

The moisture content of apricot gum samples was determined by AOAC method (AOAC, 1990) at 105 ˚C to obtain a constant weight. Finally, the moisture content was determined as follows:

Percentage of moisture (%)= (water weight in the sample/ the total weight of the wet sample) × 100

The total carbohydrate content was analyzed using phenol-sulfuric acid as the standard D-galactose. The content of uronic acids was measured using the carbazole method using D-galactose as standard. The absorbance was determined using a UV-vis spectrophotometer (Geneus 20, USA) at 490 nm and 535 nm for the total carbohydrate and uronic acid content, respectively. Ash and total fat content in apricot gum were measured based on AOAC method (AOAC, 1990). The crude protein content was measured using the Kjeldahl method and considering 6.25 as the conversion rate of nitrogen to crude protein. All experiments were repeated four times and moderate values were reported.

pH measurement

The pH of 1% solution (weight-volume) of apricot gum was measured at room temperature using a pH meter with glass electrode (Model A Z 86552, Azerbaijan).

Collect and dilute semen

This study carried out at the Agricultural Research Station of Tabriz University (38˚ 07′ N and 46˚ 29′ E) with altitude of 1567 m. At present study, 5 Ghezel rams (3-4 years old Native) were used for sperm collection. The rams were kept in an indoor hall during the research period and fed with the same diet. They were adapted to the sperm collection process for two weeks before sampling. Sampling was performed twice a week by an artificial vagina during the breeding season (from July 6 to the end of December) and were replicated 5 times for each ram. An artificial vagina filled with water at about 48 -50 ˚C was used for semen collection.

An artificial vaginal cover was used to protect the sperm from light and cold shock. After transferring the samples to the laboratory, a series of initial evaluations such as the devoid of urine contamination, semen volume, wave motion, percentage of total mobility and progressive were measured in fresh semen using a phase-contrast microscope with a magnification of 400×. Viability rate and abnormal sperm percentage (using Eosin-Nigrosine staining and 400×magnification phase-contrast microscope), and sperm concentration was determined using a tuma haemocytometer under a phase-contrast microscope with 400 × magnification. Semen pH was measured by paper strips of pH meter. Samples that met the required standard indices (concentration above 2.5 billion sperm, progressive movement above 70% and abnormal sperm less than 10%) were diluted. Tris-based diluent was prepared with 2.71 g of Tris (64271 merck, Germany), 1.4 g of fructose (0043 Dae jung chemical, Korea), 1 g of citric acid monohydrate (64291 Appli chem Gmbh, Germany), 100000 IU of penicillin and 100 mg of streptomycin in 100 ml of sterile distilled water. Then 73% of the prepared solution was mixed with 20% egg yolk and 7% glycerol (1094B56815 Arman sina, Iran) to prepare the diluent. Treatments consisted of a control group (without gum) and 3 treated groups were pure and sterilized apricot gum with concentrations of 10, 15 and 20 mg/ml added to the Tris-based diluent. Semen was diluted with diluents in a ratio of 1 to 10, which was considered 2.5 ml of diluent for each treatment and 6 straws (0.25 mL) to determine the sperm quality (total motility, progressive movement, survival, hypo osmotic swelling test and abnormality) and two straws (0.5 mL) for MDA measurement (Imv technologies, frence) were filled and the heads of the straws were closed with hematocrit paste. The straws were placed in screwed glass tubes in water at 37 ˚C and transferred to the refrigerator and refrigerated for 1.5-2 hours until the temperature slowly reached 5 ˚C (slow cooling method). Then, for freezing, they were kept in nitrogen vapor for 4 minutes at 4-5 cm above the nitrogen level and then immersed in liquid nitrogen. Frozen samples were stored for 2 months and thawing was performed on the first day (8 hours after freezing), 15, 30, 45 and 60. Defrosting of each semen sample diluted with apricot gum at concentrations of 10, 15 and 20 mg/mL in diluent and control treatment on each of the first days (8 hours after freezing), 15, 30, 45 and 60 were performed after freezing process. The parameters of total motility, progressive movement, survival, hypo osmotic swelling test (HOST), abnormality on all days of defrosting and MDA were measured only on day 60 after freezing.

Measurement of thawed sperm traits

Sperm thawing was performed at 38 ˚C for 20 seconds and the thawed semen was poured into 1.5 mL microtubes and then placed in a 37 ˚C water bath for 5 minutes for adaptation. To evaluate sperm total motility and progressive motility, 10 microliters of thawed semen was poured into a preheated slide (37 ˚C) and was spread evenly, and then using a phase-contrast microscope with Magnification 400 ×, and by examining 5 areas of the slide percentage of total motility was determined.

Also, to determine the amount of progressive motility, the same sample is used that was placed on the 37 ˚C slide to determine the total motility sperm. To do this, 200 sperm

from several different points of the slide were counted using a phase-contrast microscope with a magnification of 400× and the ratio of sperms with progressive motility was calculated. For example, if the total motility was 80% and 180 sperm out of 200 sperm had progressive motility, the amount of progressive motility was calculated as follows (WHO, 2010).

Progressive motility rate: (180×80) / 200 = 72%

Sperm viability was assessed using staining with eosin nigrosin (1.67 g water-soluble eosin, 10 g water-soluble nigrosin, 2.9 g sodium citrate and distilled water up to 100 ml). For this purpose, 10 microliters of semen at 37 ˚C with 10 microliters of eosin nigrosin at 37 ˚C gently was speared on a clean and heated slide (37 ˚C). 200 sperms from 5 different areas of the smear were counted and evaluated by phase-contrast microscope with a magnification of 400×. Dead sperm are stained due to defects in the acrosome at the head of the sperm, but live sperm are not stained due to the health of the acrosome and remain colorless. Based on that, the viability rate was estimated. Also sperm abnormalities were determined in stained smear. Having a large head, small head, narrow head, no tail, no head, twisted tail, broken neck, broken middle, having abnormal acrosome and cytoplasmic droplets were known as abnormal sperm and the percentage of abnormal sperm was also determined (Evans and Maxwell, 1987).

Hypo osmotic swelling test evaluation (HOST): The HOST test can be used to assess the functional integrity of sperm membranes because sperm swell due to water invasion despite biochemically active membranes (Jeyendran *et al.* 1984). This is seen in the tail of sperm (Takahashi *et al.* 1990) because the plasma membrane around the tail is looser than the membrane around the head (Jeyendran *et al.* 1984). HOST is a simple, inexpensive and easy-to-use technique for testing of sperm membrane integrity (Correa *et al.* 1994). To evaluate the HOST test, 10 µL of thawed sperm was incubated with 100 µL of HOST solution (0.9 g fructose and 0.49 g sodium citrate in 100 mL sterile distilled water) at 38 ˚C for 30 minutes. Then 10 µL of the solution was spreaded on a warm slide at 37 ˚C. Sperms that were exposed to the hypoosmolar solution and the fluid entered the cell through the plasma membrane, healthy sperms that had an active membrane swollen from the tail area and the tail twisted and unhealthy sperms did not swell and the tail was straight and so on. The order of percentage of sperm plasma membrane integrity was determined.

Measurement of malondialdehyde (MDA) concentration

MDA is an important indicator of membrane damage and one of the toxic substances produced by the increase of reactive oxygen species. Malondialdehyde concentration was measured using reaction with thiobarbituric acid. Mix 1 ml of thawed sperm with 2 mL of trichloroacetic acid in a sterile tube and then add 1 mL of butylated hydroxytoluene with 1 mL of EDTA to the solution and then sperm tubes for centrifuged at 1200 rpm for 15 minutes. After centrifugation, 1 mL of the above clarified solution with 1 mL of 0.67% thiobarbituric acid (TBA) was poured into the tubes and placed in 95 ˚C water for 20 minutes, then cooled. It was read at a wavelength of 532 nm by a spectrophotometer.

Statistical analysis

This study was performed with 4 treatments and each in 5 replications. The obtained data were analyzed in a completely randomized design using SAS (2004) software (9.4) with the help of mixed procedure. To determine the significance of the effects of apricot gum, Tukey comparative test was used and a significant difference was reported at the level of 5%. The statistical model in this research is:

$$Y_{ijkn} = \mu + D_i + T_j + Ram_k + e_{ijkn}$$

Where:
Y_{ijkn}: each observation.
μ: population mean.
D_i: effect of the type of diluent.
T_j: effect of the duration after dilution.
e_{ijkn}: effect of the residual model.

RESULTS AND DISCUSSION

According to the results obtained in this study, it was shown that apricot gum has sperm-improving properties during storage in a Tris-based diluent. Analyzes performed after different days of thawing showed that apricot gum was diluted in all three treatments at 10, 15 and 20 mg/mL per mL, total motility, progressive movement, viability and integrity. Improved plasma membrane compared to the control group and a significant difference was observed at the level of 5%.

It also significantly reduced the rate of sperm abnormalities compared to the control group. The amount of free radical production of MDA, which was measured on day 60 after thawing, was significantly reduced, which was a significant difference of 5% compared to the control group. According to the analysis of apricot tree gum, we showed that apricot gum contains carbohydrates, uronic acids, protein, moisture and ash.

Also, according to the results of previous research on the carbohydrate composition of apricot gum, it contains different proportions of monosaccharides including arabinose,

galactose, xylose, mannose, rhamnose and phenols of hydroquinone, catechol and pyrogallol. With these results, it can be said that carbohydrates have several functions in diluents. They provide an energy substrate for sperm cells during storage and maintain a dilute osmotic pressure (Yildiz *et al*. 2000), thereby reducing intracellular ice formation and preventing sperm damage during storage in liquid nitrogen (Dolti *et al*. 2016).

The collected semen samples were initially characterized in terms of their volume, percentage of viability, total motility and progressive motility percent, abnormal sperms percent, concentration and wave motion to assess their suitability for freezing (Table 2).

Table 3 presents the effect of different levels of apricot tree gum with control treatment on total sperm motility. The results showed that treatments of 10, 15 and 20 mg/mL in diluent were significantly different from the control treatment on each of the thawing days ($P<0.05$). But there was no significant difference between the three treatments of 10, 15 and 20 mg/mL in diluent at each of the times. There was also a significant difference in comparison between different thawing times within each of the treatments ($P<0.05$). Carbohydrates either provide a source of energy for the sperm cell or protect the sperm cell from the formation of intracellular ice crystals. Although only fructose is present in ram semen, spermatozoa can also metabolize glucose and mannose when these sugars are included in storage diluents and use as an energy source. Sugars which do not provide an energy source, but which act extracellularly to maintain osmotic pressure and membrane integrity, or even 'shrink' the sperm cell, may be of value for long-term liquid storage (Maxwell and salamon, 1993). Monosaccharides are more suitable than disaccharides to preserve motility of ram spermatozoa frozen in Tris-citrate extender (Yildiz *et al*. 2000).

Diluents containing arabinose are reported to give significantly better motility scores than those containing fructose (Garcia and Graham, 1989). Polysaccharides of apricot gums contained two main carbohydrates: galactose and arabinose, with xylose as minor component (Saniewski *et al*. 2002). According to research on dog sperm, Yildiz *et al*. (2000) reported that monosaccharides, especially fructose and xylose, improved total motility, viability, and healthy acrosome rate ($P<0.05$). With these results, it can be concluded that apricot gum monosaccharides such as arabinose, mannose and xylose had an important role in maintaining the motility of ram sperm during frozen storage in liquid nitrogen.In general, about 40-50% of the sperm population does not survive after freezing-thawing (Watson, 2000).

In rams, frozen thawed semen can have a large proportion (40-60%) of motile cells, although only 20-30% remains in terms of biological function (Medeiros *et al*. 2002).

According to research by Kulaksız *et al*. (2010), after storing ram sperm frozen in liquid nitrogen for 2 months, they reported the highest motility percentage of 54% and the highest viability of 59%. Also, according to research conducted by Juyena (2011) on the effect of freezing and thawing on ram sperm, they concluded that sperm motility and survival after freezing and thawing are significantly reduced and a sperm survival of approximately 50% Observed a 40% reduction in sperm motility and obtained 35-45% motile sperm in the sample after thawing, which is consistent with our results. The amount of motility reported by D'Alessandro and Martemucci (2003) showed that they observed about 60% of motile sperm after thawing using Tris-based developer material.

Table 4 shows a comparison of the percentage of progressive motility in different levels of apricot gum. Treatments of 10, 15 and 20 mg/mL in diluent at the first days, 15 and 30 of thawing were significantly different from the control treatment ($P<0.05$). On day 45 of thawing, treatments of 10 and 15 mg/mL in diluent were significantly different from the control treatment ($P<0.05$), but there was no any significant difference between the treatment of 20 mg/mL in diluent with the control treatment. Also, on the 60th day of thawing, any significantly differences were not observed between the treatments of 10, 15, 20 mg/mL in diluent and control.

Gums can be classified as pathological products consisting of calcium, potassium, and magnesium salts of complex substances called "polyurionides" (Prajapati *et al*. 2013). Polyuronides are polysaccharides that contain uronic acids, for example, D-galacturonic acid or D-glucuronic acid. Uronic acids are always in the form of pyranose, giving macromolecules their acidic properties as well as their ability to store water and thus form gels. In general, all natural gums are heterogeneous and, by hydrolysis, produce simple sugar units such as arabinose, galactose, glucose, mannose, xylose, or uric acids (Rana *et al*. 2011). Gums are typically more or less sticky in nature and are translucent and amorphous substances, having high molecular weights, generally composed of monosaccharide units joined by glucosidic bonds (Khorsha *et al*. 2016). Perhaps due to the sticky of the gum, the rate of progressive movement was less than the total motility, but in the first days of thawing until day 45, a significant difference was observed between the treatments of 10, 15 and 20 mg/mL in diluent with the control treatment ($P<0.05$).

Table 2 Evaluation of the quantity and quality of ram sperm before dilution

Trait	N	Mean	SD	Minimum	Maximum
Volume (mL)	25	1.08	0.165	0.8	1.5
Wave motion (0-5)	25	4.9	0.307	4	5
Total motility (%)	25	94.42	4.432	82.2	98.5
Progressive motility (%)	25	91.69	7.022	71.9	97.9
Viability (%)	25	96.801	2.261	91.29	99.38
Concenter ($\times 10^9$)	25	6.36	1.947	2.7	9.634
Abnormality (%)	25	3.76	0.859	2.45	5.39
pH	25	6.99	0.194	6.6	7.3

SD: standard deviation.

Table 3 Comparison of the percentage of total motility between apricot gum groups and control group in different

Thawing days	10 mg/mL	15 mg/mL	20 mg/mL	Control
0	76.00^{Aa}	75.95^{Aa}	79.40^{Aa}	64.55^{Bb}
15	68.10^{Ba}	67.90^{Ba}	72.25^{Ba}	57.35^{Ab}
30	62.65^{Ca}	62.40^{Ca}	64.90^{Ca}	52.30^{Db}
45	56.65^{Da}	56.30^{Da}	58.85^{Da}	47.75^{Cb}
60	50.85^{Ea}	50.65^{Ea}	52.80^{Ea}	44.30^{Fb}

$^{A, B, C, D, E:}$ in each column indicate a significant difference among the thawing days inside each group (P<0.05).
$^{a, b}$: in each row indicate a significant difference among groups at each of the thawing days (P<0.05).

Table 4 Comparison of the percentage of progressive movement between apricot gum groups and control group on different days of thawing

Thawing days	10 mg/mL	15 mg/mL	20 mg/mL	Control
0	69.07^{Aa}	68.38^{Aa}	69.65^{Aa}	57.56^{Bb}
15	58.56^{Ba}	56.43^{Ba}	56.86^{Ba}	43.82^{Ab}
30	50.12^{Ca}	49.52^{Ca}	46.69^{Ca}	38.12^{Db}
45	39.63^{Da}	41.23^{Da}	38.31^{Dab}	32.97^{Cb}
60	32.88^{Ea}	34.29^{Ea}	31.72^{Ea}	26.94^{Ea}

$^{A, B, C, D, E:}$ in each column indicate a significant difference among the thawing days inside each group (P<0.05).
$^{a, b}$: in each row indicate a significant difference among groups at each of the thawing days (P<0.05).

Table 5 shows a comparison of viability rates at different levels of apricot gum with control treatment. There was a significant difference between treatments of 10, 15 and 20 mg/mL in diluent with the control treatment (P<0.05). There is also a significant difference between the thawing times within each treatment (P<0.05). The addition of small quantities of some sugars like xylose or fructose to either yolk-citrate or milk diluents has been reported to increase the proportion of sperm that survive freezing and thawing (Garcia and Graham, 1989). According to this report, it is possible that the xylose in the gum was able to improve the survival rate of ram sperm during storage compared to the control group.

Collecting semen in farm animal species is not an easy task and some bacterial infections cannot be prevented (Aurich and Spergser, 2007; Bielanski, 2007). In ram sperm collection, semen is usually collected through an artificial vagina, which can be infected in a variety of ways, such as dead penile surface bacteria, sperm collection sites, equipment, and people. As a result, bacteria may compromise the quality of semen during storage.

To reduce these side effects, antibiotics are incorporated into ram semen diluents to prevent bacterial growth (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000). According to research by Yániz *et al*. (2010) on the isolation of ram sperm bacteria, the most isolated bacteria were *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Staphylococcus epidermis* and *Staphylococcus aureus*. These 5 bacteria were present in 97% of infected samples. However, not all bacteria can be killed. Bacteria can directly affect the reproductive performance of males, reduce motile sperm, thereby reducing viability, reduce the ability of the acrosome to react and change cell morphology, and indirectly, by producing reactive oxygen species (ROS) decreses the quality of sperm (Moretti *et al*. 2009). Phenol-derived compounds are also present in apricot gum, which include catechol, hydroquinone and pyrogallol (Babken *et al*. 2018). Polyphenols are a broad class of natural plant secondary metabolites, which are widely distributed in plants. Phenols are divided into many subgroups according to their chemical structures (including simple phenols, phenolic acids, flavonoids, lignans, flavonoligna-

ns, and tannins).

Many of their other biological activities are powerful antioxidants (Košinová *et al*. 2012). In the food industry, phenolic compounds are used as antibiotics. In addition, they have antioxidant activity. The antioxidant properties of phenolic compounds are due to the presence of structural elements such as catechol groups and hydroxyl groups that are directly involved in antioxidant activity (Socrier *et al*. 2019). It is possible that apricot gum, due to its phenolic compounds, kills bacteria that have not yet been killed by the addition of penicillin and streptomycin antibiotics, thereby reducing bacteria in the diluent medium as well as by its antioxidant activity to prevent the production of ROS.

As shown in Table 6, the comparison of the percentage of abnormality among groups in the first day, 15 and 30 thawing, any significantly difference between each of the groups 10, 15, 20 mg/mL in diluent and control group was not observed, but on 45 and 60 days of thawing, a significant difference was observed between the treatments of 10, 15 and 20 mg/mL in diluent with the control treatment ($P<0.05$).

Also, in comparing the times in the two groups of 10 mg/mL in diluent treatment and control treatment, a significant difference was observed between all times ($P<0.05$). At 15 mg/mL in diluent treatment, no significant difference was observed between the first and 15 days of thawing and between the 45th and 60th days of thawing. There was a significant difference between other thawing days ($P<0.05$). There was no significant difference in the treatment of 20 mg/mL between 45 and 60 days of thawing, but a significant difference was observed between the other thawing times in this treatment ($P<0.05$). Table 7 shows a comparison of membrane integrity among groups. There was a significant difference between treatments of 10, 15 and 20 mg/mL in diluent with the control treatment on each of the thawing days ($P<0.05$), but significantly difference was not within the gum treated groups. In comparison between thawing days, a significant difference was observed between each of the treatments in all thawing days ($P<0.05$).

In general, exudate gums are complex and branched heterogeneous polysaccharides with a combination of minor components such as proteins and phenolic (Simas *et al*. 2004). Studies conducted by Bashir *et al*. (2018) on the antioxidant properties of three exudate gums of apricot gum, gum arabic and almond gum showed that apricot gum at a concentration of 1 mg/mL inhibited the radical DPPH by 35.52% and at a concentration of 10 mg/mL inhibits hydroxyl radical (OH) by 58.31%. Apricot gum secretions have been used as medicinal drugs in Iran. In the chemical composition of this chewing gum, high content of potassium, calcium, magnesium was found (Fathi *et al*. 2016).

Hamdani *et al*. (2018) examined the properties of some exudate gums and they reported that the gum has a high antioxidant potential. Apricot gum showed higher antioxidant potential than acacia and caraway gums. Considering that the antioxidant properties of apricot gum have been proven in several studies, in this study, the antioxidant activity of apricot gum can be clearly observed, which has preserved more damage to the plasma membrane of sperm during storage than the control group. And all three treatments of apricot gum have a significant difference with the control treatment ($P<0.05$).

Comparison of MDA production between different levels of apricot gum treated groups and control group on day 60 of thawing has been showed in Table 8. There was a significantly difference between the treatments of 10, 15 and 20 mg/mL in diluent with the control treatment ($P<0.05$), but any significant difference was not observed between the treatments of 10, 15 and 20 mg/mL in diluent.

The presence of high concentrations of long chain polyunsaturated fatty acids within the lipid structure of sperm cells requires efficient antioxidant systems to defend against peroxidative damage and associated sperm dysfunction (Bucak *et al*. 2007). Furthermore, the process of semen cryopreservation produces significant amounts of ROS which may lead to impairment of sperm morphology, function, and ultimately male fertility (Khodaei *et al*. 2014).

Adding antioxidant substances to the freezing extender can reduce the oxidative stress caused by excessive ROS formation, as well as maintaining normal sperm functions associated with ROS. Hence, it is important to choose a proper antioxidant level to preserve the natural balance that exists between ROS generation and scavenging activities during cryopreservation process (Mehdipour *et al*. 2016).

Lipid peroxidation induced by reactive oxygen compounds directly damages the phospholipid components of cell membranes and produce a stable compound called MDA, which has a genotoxic effect (a substance that damages DNA). As a result, they interfere with sperm motility (Sarlos *et al*. 2002). During recent years, use of herbal antioxidants, has been gaining attention from several researchers.

For instance, the plant species such as sage, oregano, and rosemary have been tested for development of the natural antioxidant formulations in the areas of medicine and nutrition (Khodaei *et al*. 2014). Also, according to Abd El-Hamid (2019) research, adding 0.1 and 0.2 mM caffeine as an additive antioxidant has a positive effect on total motility and quality of ram sperm. Khodaei *et al*. (2014) reported that the use of 4% and 6% levels of aqueous rosemary extract as an antioxidant compared to other groups showed a higher percentage of total motility and progressive movement and plasma membrane performance ($P<0.05$). The highest viability was observed in the 6% group compared to the other groups.

Table 5 Comparison of viability rate between apricot gum groups and control group in different days of thawing

Thawing days	10 mg/mL	15 mg/mL	20 mg/mL	Control
0	85.52^{Aa}	84.67^{Aa}	88.19^{Aa}	74.9^{Bb}
15	78.63^{Ba}	76.91^{Ba}	81.29^{Ba}	67.85^{Ab}
30	72.04^{Ca}	71.4^{Ca}	75.44^{Ca}	61.94^{Db}
45	65.77^{Da}	65.68^{Da}	70.49^{Da}	56.5^{Cb}
60	60.56^{Ea}	60.35^{Ea}	63.1^{Ea}	51.45^{Fb}

$^{A, B, C, D, E:}$ in each column indicate a significant difference among the thawing days inside each group (P<0.05).
$^{a, b:}$ in each row indicate a significant difference among groups at each of the thawing days (P<0.05).

Table 6 Comparison of the percentage of abnormality between apricot gum groups and control group in different days of thawing

Thawing days	**10 mg/mL**	**15 mg/mL**	**20 mg/mL**	**Control**
0	5.12^{Aa}	5.20^{Aa}	5.24^{Aa}	5.73^{Aa}
15	7.51^{Ba}	7.07^{Aa}	7.38^{Ba}	8.77^{Ba}
30	10.39^{Ca}	10.22^{Ca}	9.94^{Ca}	12.10^{Ca}
45	13.00^{Da}	12.79^{Da}	12.24^{Da}	15.97^{Eb}
60	15.63^{Ea}	14.22^{Da}	14.11^{Da}	18.36^{Fb}

$^{A, B, C, D, E:}$ in each column indicate a significant difference among the thawing days inside each group (P<0.05).
$^{a, b:}$ in each row indicate a significant difference among groups at each of the thawing days (P<0.05).

Table 7 Comparison of the percentage of membrane integrity between apricot gum groups and control group on different days of thawing

Thawing days	10 mg/mL	15 mg/mL	20 mg/mL	Control
0	72.61^{Aa}	73.28^{Aa}	76.34^{Aa}	61.67^{Bb}
15	64.51^{Ba}	65.06^{Ba}	68.32^{Ba}	54.52^{Ab}
30	58.13^{Ca}	59.18^{Ca}	61.08^{Ca}	49.35^{Db}
45	52.18^{Da}	53.91^{Da}	54.67^{Da}	44.24^{Cb}
60	46.51^{Ea}	47.8^{Ea}	47.94^{Ea}	39.34^{Fb}

$^{A, B, C, D, E:}$ in each column indicate a significant difference among the thawing days inside each group (P<0.05).
$^{a, b:}$ in each row indicate a significant difference among groups at each of the thawing days (P<0.05).

Table 8 Comparison of the production of malondialdehyde (nmol/mL) between different levels of apricot gum with control group on day 60

Treatment	**Minimum**	**Maximum**	**Mean**	**SD**
Control	2.957	7.161	4.612^{a}	1.13
10 mg/mL	1.148	3.451	2.215^{b}	0.706
10 mg/mL	1.277	3.296	2.199^{b}	0.5009
20 mg/mL	1.058	3.709	2.077^{b}	0.659

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Low concentrations of MDA in 4% and 6% groups of rosemary extract were 2.98 and 2.74 nmol/mL, respectively, and the amount of MDA in the control group was 3.61 nmol/mL. According to the research of Atessahin *et al.* (2008) on taurine and cysteine as antioxidants, the amount of MDA measured in taurine groups with concentrations (25, 50 and 75 mM) and control group were 5.54, 9.80, 4.46 and 6.27 nmol/mL respectively and in the cysteine groups with concentrations (5, 10 and 15 mM) and the control group were 7.54, 9.02, 8.66 and 6.27 nmol/mL, respectively. The amount of MDA measured for apricot gum treatments in this study was lower than the studies performed on rosemary extract, taurine and cysteine as a hollow antioxidant, which indicates the presence of antioxidant properties in apricot gum.

Apricot gum, due to its different carbohydrates, can provide the energy needed by sperm, as well as maintain a dilute osmotic pressure and reduce cell damage by reducing the formation of intracellular ice crystals. Apricot gum can also act as an antioxidant system due to its phenolic compounds such as catechols, hydroquinone's and pyrogallols. The antimicrobial properties of phenolic compounds are due to the presence of structural elements such as catechol groups and hydroxyl groups that are directly involved in antimicrobial activity.

CONCLUSION

According to mentioned functional factors of apricot gum and obtained results from this experiment, the use of this gum in semen extender is recommended to improve the quality of frozen sperm. Apricot gum also has antioxidant activity that has been proven in several different studies and in this study has reduced the amount of free radical production of MDA compared to the control group (without apricot gum), which indicates its antioxidant activity.

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REFERENCES

Abd El-Hamid I.S. (2019). Effect of adding different levels of caffeine in the extender on some biochemical constituents, enzymatic activities and physical characteristics of chilled and frozen ram semen. *Reprod. Domest. Anim*. **54,** 225-233.

Aitken R.J. (2000). Possible redox regulation of sperm motility activation. *J. Androl.* **21(4),** 491-496.

Anand M., Baghel G. and Yadav S. (2017). Effect of egg yolk concentration and washing on sperm quality following cryopreservation in Barbari buck semen. *J. Appl. Anim. Res*. **45,** 560-565.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Atessahin A., Bucak M.N., Tuncer P.B. and Kızıl M. (2008). Effects of anti-oxidant additives on microscopic and oxidative parameters of Angora goat semen following the freeze–thawing process. *Small Rumin. Res.* **77(1),** 38-44.

Aurich C. and Spergser J. (2007). Influence of bacteria and gentamicin on cooled-stored stallion spermatozoa. *Theriogenology*. **67,** 912-918.

Babken C.N., Suren M., Naira S. and Yelena M. (2018). The phythochemical research of Armenian apricot gums (*Gummi armeniacae*). *Phcog. J*. **10,** 476-479.

Bashir M., Usmani T., Haripriya S. and Ahmed T. (2018). Biological and textural properties of underutilized exudate gums of Jammu and Kashmir, India. *Int. J. Boil. Macromol*. **109,** 847-854.

Bielanski A. (2007). Disinfection procedures for controlling microorganisms in the semen and embryos of humans and farm animals. *Theriogenology*. **68,** 1-22.

Bucak M.N., Ateşşahin A., Varışlı Ö., Yüce A., Tekin N. and Akçay A. (2007). The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen: Microscopic and oxidative stress parameters after freeze–thawing process. *Theriogenology*. **67,** 1060-1067.

Chichoyan N. (2011). Investigation and record of the apricot trees' gums raw resources of flora of the republic of Armenia. *Geo Med News*. **11,** 117-121.

Correa J.R. and Zavos P.M. (1994). The hypoosmotic swelling test: Its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology*. **42(2),** 351-360.

D'Alessandro A.G. and Martemucci G. (2003). Evaluation of seasonal variations of semen freezability in Leccese ram. *Anim. Reprod. Sci*. **79,** 93-102.

Dolti P., Moghaddam G. and Ahmadian H. (2016). Evaluation of the effects of cysteine and trehalose on long- term cryopreservation of ram semen. *J. Agric. Food Appl. Sci*. **4,** 1-6.

El-Harairy M.A., Khalil W.A., Khalifa E.I. and Saber A.A. (2018). Effect of propolis ethanolic extract supplementation to ram semen extenders on sperm characteristics, lipid peroxidation and some enzymatic activities in seminal plasma in chilled semen. *J. Anim. Poult. Prod*. **9,** 235-243.

Evans G. and Maxwell W.C. (1987). Salamons' Artificial Insemination of Sheep and Goats. Butterworths, Sydney, Australia.

Fathi M., Mohebbi M. and Koocheki A. (2016). Some physicochemical properties of Prunus armeniaca L. gum exudates. *Int. J. Biol. Macromol*. **82,** 744-750.

Garcia M.A. and Graham E.F. (1989). Development of a buffer system for dialysis of bovine spermatozoa before freezing. II. Effect of sugars and sugar alcohols on posthaw motility. *Theriogenology*. **31,** 1029-1037.

Hamdani A.M., Wani I.A., Bhat N.A. and Masoodi F.A. (2018). Chemical composition, total phenolic content, antioxidant and antinutritional characterisation of exudate gums. *Food Biosci*. **23,** 67-74.

Iravani S. (2020). Plant gums for sustainable and eco-friendly synthesis of nanoparticles: recent advances,inorganic and nano-metal chemistry. *J. Inorg. Nano-Metal. Chem*. **50,** 469-488.

Jeyendran R.S., Van der Ven H.H., Perez-Pelaez M., Crabo B.G. and Zaneveld L.J.D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *Reproduction*. **70(1),** 219-228.

Juyena N.S. (2011). Protein profiles and biochemical characteristics of semen: influence on frozen-thawed spermatozoal quality in rams (*Ovies aries*) and alpacas (*Vicugna pacos*). MS Thesis. University of Padova, Padova, Italy.

Juyena N.S. and Stelletta C. (2012). Seminal plasma: An essential attribute to spermatozoa. *J. Androl*. **33,** 536-551.

Khodaei M.M., Sharifi M., Zhandi M., Mohammadi A., Shakeri M., Soleimani M. and Zeinoaldini S. (2014). Antioxidant effect of rosemary (*Rosmarinus officinalis*) extract in soybean lecithin-based semen extender following freeze–thawing process of ram sperm. *Cryobiology*. **69,** 217-222.

Khorsha S., Alizadeh M. and Mashayekhi K. (2016). The usefulness of apricot gum as an organic additive in grapevine tissue culture media. *Adv. Hortic. Sci*. **30,** 111-118.

Khosrowbeygi A., Zarghami N., Nouroozzadeh J. and Ghaffari M. (2004). Relationship between level of lipid peroxidation markers in seminal plasma and sperm motility. *J. Reprod. Infertil.* **5(2),** 129-138.

Košinová P., Berka K., Wykes M., Otyepka M. and Trouillas P. (2012). Positioning of antioxidant quercetin and its metabolites in lipid bilayer membranes: Implication for their lipidperoxidation inhibition. *J. Phys. Chem. B*. **116,** 1309-1318.

Kulaksız R., Çebi Ç., Akçay E. and Daşkın A. (2010). The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen. *Small Rumin. Res*. **88(1),** 12-15.

Lluveras-Tenorio A., Mazurek J., Restivo A., Colombini M.P. and Bonaduce I. (2012). Analysis of plant gums and saccharide materials in paint samples: comparison of GC-MS analytical

procedures and databases. *Chem. Cent. J.* **6(1),** 115-125.

Mahmoudi M., Ebrahimzadeh M.A., Nabavi S.F., Hafezi S., Nabavi S.M. and Eslami S.H. (2010). Antiinflammatory and antioxidant activities of gum mastic. *European Rev. Med. Pharmacol. Sci*. **14,** 765-769.

Makker K., Agarwal A. and Sharma R. (2009). Oxidative stress and male infertility. *Indian J. Med. Res.* **129(4),** 357-367.

Maxwell W.M. and Salamon S. (1993). Liquid storage of ram semen: a review. *Reprod. Fertil. Dev.* **5,** 613-638.

Medeiros C.M.O., Forell F., Oliveira A.T.D. and Rodrigues J.L. (2002). Current status of sperm cryopreservation: Why isn't it better? *Theriogenology*. **57(1),** 327-344.

Mehdipour M., Daghigh Kia H., Najafi A., Dodaran H.V. and García-Álvarez O. (2016). Effect of green tea (*Camellia sinensis*) extract and pre-freezing equilibration time on the post-thawing quality of ram semen cryopreserved in a soybean lecithin-based extender. *Cryobiology*. **73,** 297-303.

Moretti E., Capitani S., Figura N., Pammolli A., Grazia Federico M., Giannerini V. and Collodel G. (2009). The presence of bacteria species in semen and sperm quality. *J. Assist. Reprod. Genet*. **26,** 47-56.

Morrow D.A. (1986). Current therapy in theriogenology2: Diagnosis, treatment and prevention of reproductive diseases in small and large animals. Saunders Company, Newberg, USA.

Motta J.P.R., Paraguassú-Braga F.H., Bouzas L.F. and Porto L.C. (2014). Evaluation of intracellular and extracellular trehalose as a cryoprotectant of stem cells obtained from umbilical cord blood. *Cryobiology*. **68(3),** 343-348.

Prajapati V.D., Jani G.K., Moradiya N.G. and Randeria N.P. (2013). Pharmaceutical applications of various natural gums, mucilage's and their modified forms. *Carbohydr. Polym*. **92(2),** 1685-1699.

Rahimi S., Abasi S., Sahari M. and Azizie M. (2013). Isolation and identification of chemical and functional properties of soluble and insoluble parts of Persian gum. *J. Food Sci. Ind*. **40(10),** 1-10.

Rana V., Rai P., Tiwary A.K., Singh R.S., Kennedy J.F. and Knill C.J. (2011). Modified gums: Approaches and applications in drug delivery. *Carbohydr. Polym*. **83(3),** 1031-1047.

Rosik J., Kubala J., Stanova M. and Lacok P. (1971). Structural properties of apricot gum polysaccharides. IV. Observation on their changes during vegetave cycle after evoked gummosis by pathogens. *Biologia*. **26,** 13-18.

Salamon S. and Maxwell W.M.C. (2000). Storage of ram semen. *Anim. Reprod. Sci*. **62(1),** 77-111.

Samaei S., GHorbani M., Sadeghi M.A. and Jafari S. (2017). Physicochemical and functional properties of apricot tree gum. *Iranian J. Food Sci. Technol*. **14,** 335-342.

Saniewski M., Ueda J., Horbowicz M., Miyamoto K. and Puchalski J. (2002). Gum in apricot (*Prunus armeniaca*) shoots induced by methyl jasmonate. *Acta Agrobot*. **54,** 27-34.

Sariözkan S., Bucak M.N., Canturk F., Özdamar S., Yay A., Tuncer P.B., Ozkan S., Sorgucu N. and Caner Y. (2012). The effects of different sugars on motility, morphology and DNA damage during the liquid storage of rat epididymal sperm at 4 ˚C. *Cryobiology*. **65(2),** 93-97.

Sarlos P., Molnar A., Kokai M., Gabor G.Y. and Ratky J. (2002). Comparative evaluation of the effect of antioxidants in the conservation of ram semen. *Acta Vet. Hung*. **50,** 235-245.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Simas F.F., Gorin P.A., Guerrini M., Naggi A., Sassaki G.L., Delgobo C.L. and Iacomini M. (2004). Structure of a heteroxylan of gum exudate of the palm Scheelea phalerata (uricuri). *Phytochemistry*. **65(16),** 2347-2355.

Singleton P. and Sainsbury D. (1981). Dictionary of Microbiology. John Wiley and Sons, New York, USA.

Socrier L., Quéro A., Verdu M., Song Y., Molinié R., Mathiron D., Pilard S., Mesnard F. and Morandat S. (2019). Flax phenolic compounds as inhibitors of lipid oxidation: Elucidation of their mechanisms of action. *Food Chem*. **274,** 651-658.

Soltanpour F. and Moghaddam G. (2013). Effects of frozen diluents on storage of ram sperm. *Int. J. Adv. Biol. Biomed. Res*. **1,** 1698-1704.

Sterbenc N., Kosec M., Bollwein H. and Klinc P. (2014). The effect of Equex STM in freezing media on post thaw motility, viability and dna integrity of frozen-thawed ram spermatozoa. *Slovenian Vet. Res*. **51,** 35-42.

Taghilou P., Rostami B., Masoumi R. and Mirzaei A.H. (2017). Effects of supplementation of the Tris-egg yolk extender with n-3 polyunsaturated fatty acids (PUFAs) on frozen-thawed ram semen characteristics. *Small Rumin. Res*. **155,** 1-5.

Takahashi K., Uchida A. and Kitao M. (1990). Hypoosmotic swelling test of sperm. *Arch. Androl*. **25,** 225-242.

Tsikas D., Rothmann S., Schneider J.Y., Suchy M.T., Trettin A., Modun D., Stuke N., Maassen N. and Frölich J. (2016). Development, validation and biomedical applications of stable-isotope dilution GC-MS and GC-MS/MS techniques for circulating malondialdehyde (MDA) after pentafluorobenzyl bromide derivatization: MDA as a biomarker of oxidative stress and its relation to 15(s)-8-iso-prostaglandin F2α and nitric oxide (NO). *J. Chromatogr. B*. **1019,** 95-111.

Umansky Z.M., Olyshansky M.I. and Frimerman M.L. (1946). *Pharmacol. Toxicol*. **2,** 63-72.

Watson P.F. (2000). The causes of reduced fertility with cryopreserved semen. *Anim. Reprod. Sci*. **60,** 481-492.

WHO. (2010). World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen. Available at: http://www.who.int/reproductivehealth/publications/infertility/9789241547789/en/.

Yániz J.L., Marco-Aguado M.A., Mateos J.A. and Santolaria P. (2010). Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15 C. *Anim. Reprod. Sci.* **122(1),** 142-149.

Yildiz C., Kaya A., Aksoy M. and Tekeli T. (2000). Influence of sugar supplementation of the extender on motility, viability and acrosomal integrity of dog spermatozoa during freezing. *Theriogenology*. **54,** 579-585.

Zhang J., Yang Z., Zhang S., Xie Z., Han S., Wang L., Zhang B. and Sun S. (2020). Investigation of endogenous malondialdehyde through fluorescent probe MDA-6 during oxidative

stress. *Anal. Chim. Acta*. **1116,** 9-15.
Zitko V., Rosik J., Brutenicova M. and Kubala J. (1965). Some structural features of apricot tree gum (*Prunus armeniaca*). *Collect. Czech. Chem. Commun*. **30,** 3501-3512.